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New species of neotropical *Rhodocollybia*

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Abstract —. Sequences from the internal transcribed spacer region of the ribosomes helped circumscribe two new *Rhodocollybia* species for neotropical America: *R. olivaceogrisea* from Costa Rica; and *R. tenuipes* from Dominican Republic and Puerto Rico. Both species are phylogenetically related to *R. butyracea*. *Rhodocollybia maculata* represents a new report for Dominican Republic.

Key words — ITS, phylogeny, *Omphalotaceae*, *Agaricomycetes*

Introduction

The genus *Rhodocollybia* Singer (*Omphalotaceae*, *Agaricomycetes*) comprises fleshy mushrooms with convex smooth pilei with crowded cream-colored lamellae, striate and slightly twisted stipes, and a pinkish spore deposit (Antonín & Noordeloos 2010). Basidiospores are at least partly, dextrinoid. However, not all *Rhodocollybia* mushrooms explicitly exhibit this entire set of characters and sometimes may be confused with closely related genera, such as *Gymnopus* (Hughes et al. 2010). In addition to detailed morphological analyses, DNA studies are very helpful determining identities of many mushrooms whose species boundaries are difficult to resolve.

Most reports for *Rhodocollybia* are from temperate regions (Antonín & Noordeloos 2010, Cooper 2014, Halling 2009, Lee et al. 2014, Lennox 1979). Studies by Halling (1989) and Mata et al. (2004) thus far provide the best accounts for neotropical America.

A recent foray in Costa Rican oak-dominated forests confirmed a suspected new species of *Rhodocollybia*. Previous studies of Caribbean material also suggested new records. Here we propose two new neotropical species: *R. olivaceogrisea* from Costa Rica and *R. tenuipes* from Dominican Republic

Table 1. *Rhodocollybia* and outgroup sequences used in the ITS phylogenetic analysis. New sequences are set in bold font.

Taxon	GenBank no.	Voucher	Reference
Lentinula raphanica (Murrill) J.L. Mata & R.H. Petersen	AY016441	TFB 9156	Mata et al. 2001
Gymnopus luxurians (Peck) Murrill	KJ416241	TFB 14107	Petersen & Hughes 2014
R. amica J.L. Mata & Halling	AF505754	TFB 9920	Mata et al. 2004
R. butyracea (Bull.) Lennox	GU318386	TFB 13006	Hughes et al. 2010
	KJ609163	SFC20120821-75	Lee et al. 2014
	AY313290	TFB 8801	Mata et al. 2004
	AY313293	TFB 7452	Mata et al. 2004
	DQ444317	OKM27562	Mata et al. 2007
	AY313292	TFB 8250	Mata et al. 2004
R. dotae J.L. Mata & Halling	AF505758	REH 7007	Mata et al. 2004
R. laulaha Desjardin et al.	GU369944	SFSU: DED6393	Keirle et al. 2010
R. lignitilis J.L. Mata & Halling	AF505753	REH 7907	Mata et al. 2004
R. maculata	**KT205402**	TFB 11720	This paper
	AY313296	TFB11045	Mata et al. 2004
	AF505756	TFB 9605	Mata et al. 2004
	GU947368	BRNM 707085	Antonín & Noordeloos 2010
	GU947370	BRNM 699408	Antonín & Noordeloos 2010
	GU947369	BRNM 714632	Antonín & Noordeloos 2010
R. olivaceogrisea	**KT205399**	JLM 2175	This paper
	KT205400	CLO 4368	This paper
R. pandipes Halling & J.L. Mata	**KT205401**	JLM 2251	This paper
	AY313295	TFB 9680	Mata et al. 2004
	AF505752	TFB 11014	Mata et al. 2004
	AY313294	TFB 7899	Mata et al. 2004
R. prolixa var. *distorta* (Fr.) Antonín et al.	AF505748	EFM1403	Mata et al. 2004
R. purpurata (G. Stev.) J.A. Cooper	KJ61902	PDD:95837	Cooper 2014
R. tenuipes	AY313288	TFB 11707	Mata et al. 2004
R. tablensis J.L. Mata & Halling	AF505755	EN2066	Mata et al. 2004
R. turpis (Halling) Halling	AF505749	FB10077	Mata et al. 2004
R. unakensis (Murrill) Halling	AY313298	TFB 10482	Mata et al. 2004

and Puerto Rico. Phylogenetic analysis nests both species in a clade with *R. butyracea*. *Rhodocollybia maculata* is a new report for the Dominican Republic, and it and *R. tenuipes* are the first *Rhodocollybia* records from this country.

Materials & methods

Morphology

Basidiomata colors in capital alphanumeric codes in square brackets are from Kornerup & Wanscher (1978) and small-cap codes are from Kelly (1965). Preparation for tissues and basidiospores for microscopy observations and descriptive abbreviations are as in Mata et al. (2004). Line drawings were generated using a drawing tube attached to an Olympus CX41 microscope.

Phylogeny

DNA extraction and PCR protocols are as those used in Mata et al. (2004). Ribosomal ITS-4 and ITS-5 primers were used to amplify the internal transcribed region (ITS) of nuclear DNA (White et al. 1990). PCR products were purified with Qiaquick PCR Purification Kit (Qiagen®). Purified PCR products were sequenced at Macrogen (MD, USA). Sequence files were first edited with Geneious R8 (Geneious 2014) and then blasted against NCBI nucleotide databases. Selected *Rhodocollybia* sequences were downloaded together with one of *Lentinula raphanica* and *Gymnopus luxurians,* which were used as the outgroup (Table 1). The matrix was aligned with MAFFT (Katoh et al. 2002). Maximum likelihood phylogenetic tree was calculated with PhyML (Guindon & Gascuel 2003) using 1000 bootstrap replications and supported with posterior probabilities using Mr. Bayes (Huelsenbeck & Ronquist 2001). All programs were run with Geneious R8.

Abbreviations

Country abbreviations are as follow: Costa Rica (CRC), Czech Republic (CZR), Dominican Republic (DOM), Italy (ITA), Korea (KOR), Mexico (MEX), New Zealand (NZL), Sweden (SWE), and United States (USA). Herbarium acronyms where collections are deposited are according to Thiers (2015).

Results

Phylogeny

ITS sequences of *R. olivaceogrisea* nest in a clade with those of *R. butyracea* (a well-known species in temperate regions), but its closest relative appears to be *R. pandipes* from Costa Rica (Plate 1). Percent identity between these two species is 95.39% based on the distance matrix. Sequences from both *R. olivaceogrisea* collections are 99.73% identical, with one representing a collection from the Talamanca Mountains and the other from the Central Volcanic Range of Costa Rica. The *R. tenuipes* sequence from the Dominican Republic also nests in the clade with *R. olivaceogrisea* and *R. butyracea* but is

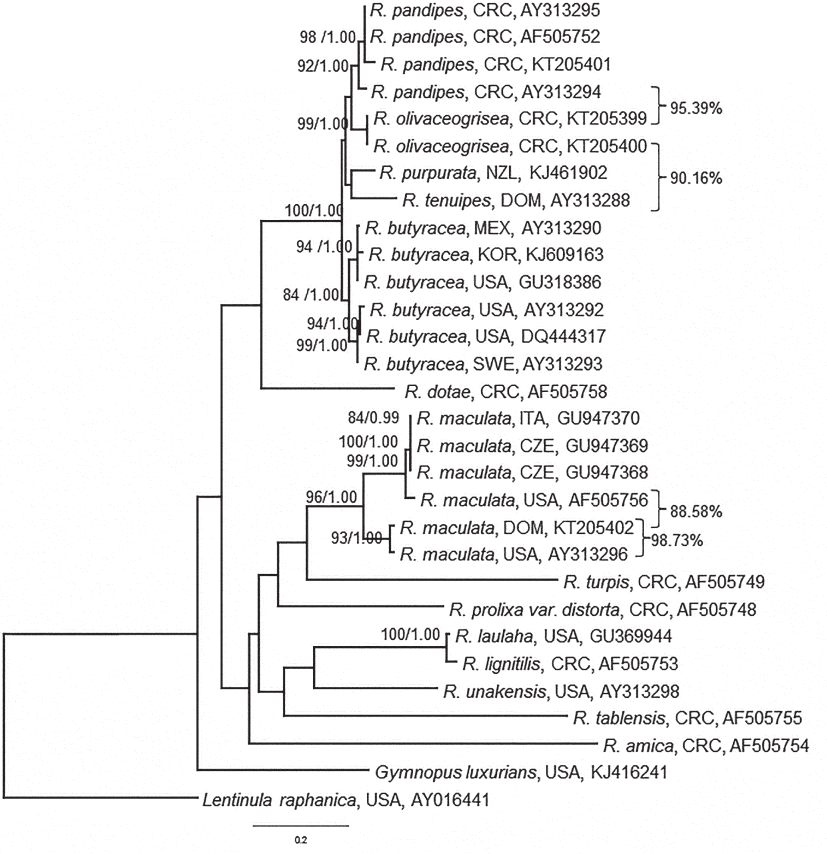

Plate 1. ITS maximum likelihood phylogeny for neotropical *Rhodocollybia* species. Branch support is presented as: "bootstrap >80%"/"posterior probability". Numbers after braces are percent identity based on distance matrix. Scale bar is for number of substitutions per site.

weakly supported. This sequence was uploaded in GenBank as *R.* aff. *pandipes* (Mata et al. 2004) and its phylogenetic placement was similar as in this study. A sequence obtained from a Dominican Republic collection is 98.73% identical to *R. maculata* from Oregon, USA. Although there are two subclades within *R. maculata,* there is not enough data to draw vicariance conclusions, mainly due to insufficient sampling. A newly generated sequence for *R. pandipes* from the Central Volcanic Range in Costa Rica is added in the phylogeny to document range extension.

Taxonomy

Rhodocollybia olivaceogrisea J.L. Mata & Ovrebo, **sp. nov.** Plate 2

MycoBank MB 812967

Differs from other *Rhodocollybia* by its green to grayish colored pilei and from *R. pandipes* by its olive to grayish stipes.

Type: Costa Rica, Province Cartago, County Oreamuno, District Potrero Cerrado, vicinity of San Juan Chicuá, at Volcán Irazú National Park, Sector Prusia, along El Roble Trail, 9°57.92′N 83°52.29′W, 2950 m a.s.l., 5.Aug.2014, col. J.L. Mata JLM 2175 (**Holotype**, USAM-F00608; **isotype**, USJ; GenBank KT205399).

Etymology: from Latin *olivaceus* for olive and *griseus* for gray.

Pileus (10–)35–45(–50) mm broad, broadly convex, to becoming almost plane, shallowly umbonate; surface glabrous, dull or shiny, waxy or greasy to the touch, when young greenish gray [3E3, 2E2, 3E2], olive brown [3C3] with grayish hues, remaining dark olive [3F6] at umbo, grayish olive [3D3] when hygrophanous, losing greenish color with age to drab grayish yellow [90 gy.y–91 d.gy.y]; margin decurved, finely crenulated, pale, translucent. Flesh 2–4 mm thick at center, translucent, watery gray, off-white when hygrophanous. Odor pleasant; taste nondescript. Lamellae adnexed, deeply adnexed to free, close (1–2/mm), 1.4–5 mm broad, white when young, in age becoming off-white to cream [2A2–3A2], not discoloring; margin entire or uneven to irregularly wavy (not serrate); two tiers of lamellulae of different lengths. Stipe (40–)80–100 × 4–7 mm at mid stipe, central, ± equal to enlarged gradually towards base and tapered towards apex, ± flexing; surface glabrous, not ridged, drab olive above, ± concolorous to pileus, and becoming olive brown, like pileus, or grayish buff at base; base rounded; consistency very fragile; interior hollow. Whitish mycelium scattered on stipe base.

Basidiospores 5.5–9 × 3.5–4.5 µm (n = 40, x = 7.2 × 3.8 µm, Q = 1.63–2.33 Qx = 1.88), ellipsoid in side view and profile, smooth, dextrinoid; wall thin. Pileipellis a cutis, composed of radially oriented hyphae, 2–6 µm diam, with occasional clusters of semi-erect terminal hyphae, 30–40 × 2–4 µm, mostly with obtuse endings, some with lobes. Pileus trama composed of radially oriented, interwoven hyphae, 8–20 µm, hyaline, thin-walled; refractive hyphae, 4–6 µm, occasionally present. Lamellar trama regular, hyphae 4–16 µm diam, ± interwoven, thin-walled. Hymenium composed of basidioles, 15–20 × 3–6 µm, and tetra-sterigmate basidia, 23–30 × 6–8 µm, mostly club-shaped. Pleurocystidia absent. Lamellar margin sterile. cheilocystidia, 20–35 × 5–9 µm, club-shaped to cylindrical, with obtuse apex, frequently with diverticula; abundant. Stipe cutis and trama parallel; epicutis hyphae 2–4 µm in diam, context hyphae 4–16 µm in diam, thin-walled. Clamp connections present in all hyphae.

Plate 2. *Rhodocollybia olivaceogrisea.* A) basidiomata (JLM 2175); B) basidiospores; C) basidia and basidioles; D) cheilocystidia. Scale bar: B–D = 10 µm.

Ecology clustered or scattered, some in cespitose bundles, in thick leaf litter layer, under oak.

Additional specimens examined: **COSTA RICA**, **Province San José**, County Dota, vicinity of San Gerardo, along road to San Gerardo, 500 m. S. of Interamerican Highway, Km 80, 9°36′13″N 84°47′26″W, 2.Jun.2004, col. C Ovrebo CLO 4368 (NY, USJ).

Commentary– This species resembles *R. butyracea* in the smooth and greasy aspect of the pileus, the swollen stipe, and spore dimensions. But it differs in its gray-olive to brown-olive colors, and in Costa Rica no other *Rhodocollybia* has these field characters. Its closest resemblance is to its Costa Rican sister taxon *R. pandipes*, which is easily distinguished by its bent stipe that soon develops reddish-colors. A tawny olive coloration is reported in pilei of North American *R. lentinoides* (Peck) Halling, but its lamellae become distinctly serrated in time (Halling 2009). The phylogenetically related *R. tenuipes* can be distinguished from *R. olivaceogrisea* by its overall brown pileus colors and a slender stipe that does not develop a strongly swollen base. Basidiomata of *R. olivaceogrisea* do not develop any spots, a field character reported for *R. turpis*, *R. dotae*, and *R. laulaha* [= *R. lignitilis*] in Costa Rica. Its dextrinoid spores set it apart from *R. amica* and *R. tablensis* (Mata et al. 2004).

Rhodocollybia tenuipes J.L. Mata, T.J. Baroni & K.W. Hughes, **sp. nov.** Plate 3

MycoBank MB 812968

Differs from *R. butyracea* by its slender and straight stipe with a striate surface, its less red pileus colors, and its longer spores.

Type: Dominican Republic, Province Santiago, Las Placetas, near Los Montones Arribas, vicinity of San José de los Matas, 19°14′N 70°53′W, 8.Jan.2003, col. EA Grand TFB 11707 (**Holotype**, TENN 59546; GenBank AY313288).

Etymology: from Latin *tenuis* for thin, and *pedes* for foot.

Pileus 10–65 mm diam, convex to broadly convex, becoming broadly campanulate or plane and then uplifted, umbonate, sometimes slightly depressed at disc; surface glabrous, smooth, slightly hygrophanous, burnt sienna [7D8], dull red-brown [5–6D5], cinnamon brown [6E6–7], paler on some [near 5C5–6]; margin curved to uplifted or undulating, incised, cream. Flesh watery cinnamon to cream, thin. Odor none or pungent-spicy, taste unpleasant, bitter. Lamellae adnate to adnexed, crowded, 1–4 mm broad, light orange [5A3], cream [5A2], pale cream [4A3] or creamy tan [4A3–5B3], now cream [3A5–4A4]; margin entire, even, concolorous. Stipe 30–80 × 3–9 mm, central, equal to flattened in some parts, somewhat twisted; surface glabrous to pubescent, with striations running entire length, light orange [5A3], pale cinnamon [5C4–5B3], whitish near apex; base slightly swollen in some; interior hollow.

Plate 3. *Rhodocollybia tenuipes*. A) basidiomata (TJB 8803); B) basidiospores; C) basidia and basidioles; D) cheilocystidia. Scale bars: A = 10 mm; B–D = 10 µm

Basidiospores in mass cream [4A2–3]; 5.5–8.8 × 3–4.4 µm (n = 4/70, x = 6.8 × 3.4 µm, Q = 1.64–2.33, Qx = 2.00), lacrymoid in side view, ellipsoid in profile, hyaline, inamyloid or dextrinoid (pale reddish-brown to red-brown); wall smooth, thin to slightly thickened in dextrinoid spores, retracted endosporium in some. Pileipellis a simple cutis; hyphae ≤5 µm diam, cylindrical, repent, radially oriented, somewhat interwoven and gelatinized; wall thin. Pileus trama composed of interwoven hyphae, ≤20 µm diam; refractive hyphae present. Lamellar trama irregular, hyphae ≤14–20 µm diam, long and cylindrical to short and barrel-shaped. Hymenium composed of basidia, 21–26 × 4–6 µm, clavate, four-sterigmate, some dextrinoid; and basidioles 16–24 × 3–5 µm, clavate. Pleurocystidia absent. Lamellar margin sterile. Cheilocystidia 20–32 × 4–7 µm, clavate to broadly clavate, or slender, some flexuous, apically obtuse, nodulose to lobed; abundant; wall thin. Stipe cutis and trama parallel; hyphae of epicutis, 2–6 µm diam, loosely interwoven, forming a mat, with terminal cells ≤50 × 5 µm, long clavate, some lobed or diverticulate, mostly prostrate, some semi–erect; hyphae of trama 6–16 µm diam, cylindrical; wall thin. Hyphae in all tissues inamyloid and with clamp connections present.

Ecology cespitose on soil, well-decayed wood (like loam); in *Pinus* vegetation.

Additional specimens examined: **DOMINICAN REPUBLIC**, **Province La Vega**, above Manabao, Cruz farm, 21.Nov.1999, coll. T Amstrong TA 265 (CORT 13982); **Province San Pedro de Macoris**, Corrizal Cordillera, 18°52′43″N 69°37′26″W, 14.Jan.2003, col. EA Grand, TFB 11725 (TENN 59554). **PUERTO RICO**, **Municipality Luquillo**, Ridge above Sabana chicken farm, 18°21′03″N 65°43′50″W, 8.Jun.1998, col. TJ Baroni TJB 8803 (CORT 13983).

Commentary– Fruiting bodies of *R. tenuipes* are similar to *R. butyracea* in color but have slender stipes. Spore dimensions are similar to American reports for *R. butyracea* (Halling 2009) but larger than those cited for European specimens (Antonín & Noordeloos 2010). Basidiomata of *R. tenuipes* lack the bulbous bent stipe base so characteristic of *R. pandipes*, and colors are more reddish-brown than are seen for *R. pandipes*. However, the spore and cystidia dimensions are similar to *R. pandipes*. *Rhodocollybia olivaceogrisea* differs from *R. tenuipes* by its overall olive gray-colored basidiomata. *Rhodocollybia tenuipes* was collected under *Pinus*, a plant genus not indigenous to Costa Rica. DNA extraction from vouchers TA 265 and TJB 8803 (and subsequent PCR intended to obtain a more robust *R. tenuipes* clade) was unsuccessful.

Rhodocollybia maculata (Alb. & Schwein.) Singer, Schweiz. Z. Pilzk. 17: 71. 1939.

Basidiomata of *Rhodocollybia maculata* from the Dominican Republic have pilei that become scrobiculate and cream-colored, lamellae that are

adnexed, crowded, and cream-colored, and stipes with a slightly swollen and bent base, collected under pine duff, or near roots of *Pinus occidentalis*. Micromorphological characters are typical of *R. maculata*, with abundant cheilocystidia 12–40 × 4–8 µm, clavate to somewhat lobed, or slender and flexuous, apically clavate, furcate or mucronate. Basidiospores 5.2–6.8 × 3.2–4.4 µm (n = 3/55, x = 6.2 × 3.9 µm, Q = 1.40–1.89, Qx = 1.56), short lacrymoid in side view, broadly ellipsoid in profile view, hyaline, inamyloid or dextrinoid (rosy brown to pale orange-brown); wall smooth, thin to slightly thickened in dextrinoid spores.

Specimens examined: **DOMINICAN REPUBLIC, Province Santiago**, Las Placetas, near Bao River, 19°14′N 70°53′W, 13.Jan.2003, col. EA Grand TFB 11720 (TENN 59804); Ebano Verde, trail from Arroyazo to Loma de la Sal, 19°03′03″N 70°32′46″W, 31.Aug.2003, col. O Perdomo, M Quirico, A Marmolejo, R Concepción MQ 209 (DR 3020), MQ 212 (DR3021).

This represents a first report of *Rhodocollybia maculata* for Dominican Republic.

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